Koisio Technology-Produced Water Significantly Decreased Inflammation and Multiple Injuries in Mouse Model of Dextran Sulfate Sodium Salt-Induced Acute Colon Inflammation

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Abstract

Inflammation is one of the crucial pathological factors of numerous diseases. It is critical to search for new strategies to decrease inflammation-produced damage. Our previous study has reported that Koisio technology-produced cell culture media produced increased antioxidant capacity of cell cultures. Since oxidative stress plays a significant role in inflammation-produced tissue injury, in our current study we used a mouse model of acute colon inflammation to test our hypothesis that Koisio technology-produced water may decrease inflammation-produced tissue damage. Our study has obtained evidence supporting the hypothesis: First, Koisio technology-produced water significantly attenuated inflammation-induced shortening of colon length in the mouse model of Dextran Sulfate Sodium salt (DSS)-induced acute colon inflammation; second, Koisio technology-produced water significantly attenuated colon inflammation-induced increase in DAI in the mouse model; third, Koisio technology-produced water significantly attenuated colon inflammation-induced increase in the Spleen Index in the mouse model; and fourth, Koisio technology-produced water significantly attenuated the increases in the myeloperoxidase (MPO) activity and the Eosinophil peroxidase (EPO) activity in the mouse model. Collectively, our study has provided novel evidence suggesting that Koisio technology-produced water can decrease inflammation-induced tissue damage in the mouse model of acute colon inflammation.

Keywords: Inflammation; Colon damage; Colon length; Spleen index; Mouse model

Introduction

Inflammation is one of the key pathological factors in numerous diseases including stroke and cancer [1, 2]. Chronical inflammation is also one of the major causes of cancer [3-5]. Since long-term administration of anti-inflammation drugs is costly, which usually produces toxic side effects, it is critical to find novel, non-toxic, economic and simple approaches that can decrease inflammation-produced tissue injury.

Our previous study has suggested that Koisio technology-produced cell culture media can enhance the antioxidant capacity of cell cultures [6]. It is established that one of the key pathological effects of inflammation originates from inflammation-induced oxidative stress, e,g., the neutrophil myeloperoxidase (MPO) is a resource of potent reactive oxygen species (ROS) [7, 8]. Therefore, we proposed our hypothesis that Koisio technology-produced water might lead to decreased inflammation-produced tissue injury. In this study, we reported our findings showing that Koisio technology-produced water can produce decreases in the inflammation and the inflammation-induced tissue injury in the mouse model of DSS-induced acute colon inflammation.

Materials and Methods

Materials

All chemicals were purchased from Sigma (St. Louis, MO, USA) except where noted. Dextran Sulfate Sodium salt (MW: 36 – 50kDa) was purchased from MP Biomedicals (Santa Ana, CA, USA). Male C57BL/6SLACBL/6Slac mice were purchased from SLRC Laboratory (Shanghai, China).

Methods

Animal model of acute colon inflammation

Male C57BL/6Slac mice at the weight between 18-24 g were administered with Koisio technology-produced water, which was produced as described previously [6], or regular water for 10 days. The experiments were conducted as described previously [9]. The mice were then administered with 3% (w:v) Dextran Sulfate Sodium salt (DSS) (0216011080, MP Biomedicals) in Koisio technology-produced water or dH_2O for 7 - 10 days. Mice were inspected daily and body weight as well as solution consumption were recorded. For determinations of the blood in feces, occult blood test (C027-1-1, Nanjing Jiancheng Bioengineering Institute, China) was used. Criteria for the scores were shown in Table 1. Disease Activity Index (DAI) was calculated by summing up the scores of all indexes of each day [10]. On the final day, mice were sacrificed and the colons as well as the spleens were obtained. The lengths of colons were measured before washing and freezing. The spleens were stored in saline on ice for testing.

Table 1. Criteria for DAI Scores

Score	%Body weight loss	Diarrhea	Bleeding (Occult blood test)
0	⩽1%	Normal	Negative
1	1-5%	Softer stool	Positive
2	6-10%	Softer stool	Positive
3	11-15%	Watery stool	Visual Blood
4	⩾15%	Watery stool	Visual Blood

Determinations of Colon Length

On the final day, colons were excised after mice were sacrificed. The colons were stored in saline on ice before measurement. After all colons were collected, colons and a measuring scale were placed on white surface. The ImageJ was used to measure the length of colons after using a camera to take photos.

Determinations of Spleen Index

On the final day, whole spleens from mice were harvested. The spleens were stored in saline on ice. After all spleens were collected, the wet weight was measured by analytical balance (Mettler Toledo AL104. Shanghai, China). The spleen index was calculated as the wet weight of the spleen (mg) divided by the final body weight (g) [11].

MPO Assay

MPO assay was conducted as described previously[10, 12]. The colon was weighted and placed separately in tube containing 0.5% hexadecyltrimethylammonium bromide in a 50 mM potassium phosphate buffer (4.35 g of dibasic potassium phosphate and 3.4 g of monobasic potassium phosphate in 1 L of dH_2O, pH=6.0). The ratio of colon to buffer was 50 mg/ml. The colons were homogenized and centrifuged at 14,000 × g, 4 °C for 5 min. The supernatant was collected for test. Before measuring the plate,

10 µl supernatant of each sample was combined with 200 µl of an *o*-dianisidine solution (0.167 mg/mL *o*-dianisidine dihydrochloride, 0.0006% hydrogen peroxide in 5mM potassium phosphate buffer pH=6.0). Changes in absorbance were measured at 460 nm and recorded at 30 s intervals for 5 min.

EPO Assay

EPO assay was conducted as described previously [13, 14]. The homogenization buffer was same as MPO assay [15]. The colons were homogenized until no large tissue exist. The solution was centrifuged at 14,000 × g, 4 °C for 5 min and the supernatant was collected for test. The reaction solution consists of 0.1 mM *o*-phenylenediamine dihydrochloride in 0.05M Tris-HCl containing 0.1% Triton X-100 and 1 mM hydrogen peroxide. Fifty µl supernatant of each sample was combined with 100 µl of reaction solution and the plate left at room temperature for 30 min, following by addition of 50 µl of 4 M sulphuric acid to stop reaction. The absorbance of each sample was determined at 492nm.

Statistical Analysis

Data were presented as mean ± SEM and analyzed by one way analysis of variance (ANOVA) followed by Student-Newman-Keuls *post hoc* test. *P* values less than 0.05 were considered statistically significant.

Results

1.Koisio technology-produced water significantly attenuated the shortening of colon length, increases in DAI, and decreases in Spleen Index in the mouse model of DSS-induced acute colon inflammation

DSS induced significant shortening of the colon in the mouse model of DSS-induced acute colon inflammation, which was significantly attenuated in the mice that had drunk Koisio technology-produced water (Fig. 1). DSS was found to induce a significant increase in DAI, which was significantly attenuated in the mice that had drunk Koisio technology-produced water (Fig. 2). DSS also induced a significant increase in the Spleen Index, which was significantly attenuated in the mice that had drunk Koisio technology-produced water (Fig. 3).

2.Koisio technology-produced water significantly attenuated the increases in MPO activity and EPO activity in the mouse model of DSS-induced acute colon inflammation

DSS induced significant increases in MPO activity, which was significantly attenuated in the mice that had drunk Koisio technology-produced water (Fig. 4). DSS also induced significant increases in EPO activity, which was significantly attenuated in the mice that had drunk Koisio technology-produced water (Fig. 5).

Discussion

The major findings of our study include: First, Koisio technology-produced

water significantly attenuated inflammation-induced shortening of colon length in the mouse model of Dextran Sulfate Sodium salt (DSS)-induced acute colon inflammation; second, Koisio technology-produced water significantly attenuated colon inflammation-induced increase in DAI in the mouse model; third, Koisio technology-produced water significantly attenuated colon inflammation-induced increase in Spleen Index in the mouse model; and fourth, Koisio technology-produced water significantly attenuated colon inflammation-induced increases in the MPO activity and EPO activity in the mouse model.

Our previous study has reported that the cell cultures that grew in Koisio technology-produced cell culture media had significant higher antioxidant capacity [6]. Since one of the key pathological effects of inflammation originates from inflammation-induced oxidative stress [7], we proposed the hypothesis that Koisio technology-produced water might produce decreased levels of inflammation-produced tissue injury. Our study has provided evidence supporting this hypothesis: Our study has indicated that Koisio technology-produced water can decrease three indices of tissue damage in the mouse model of DSS-induced acute colon inflammation, including shortening of colon length, increased Spleen Index, and increased DAI.

Our study has also found that Koisio technology-produced water significantly attenuated colon inflammation-induced increases in the MPO activity and EPO activity in the mouse model. Since increased MPO activity and increased EPO activity are two important indices of inflammation, our study has suggested that Koisio technology-produced water could decrease the inflammation in the mouse model. However, it is

warranted to investigate the mechanisms underlying the inhibitory effects of Koisio technology-produced water on the activities of the enzymies.

Our previous study has reported that Koisio technology-produced cell culture media had significant antioxidant capacity [6], we proposed that Koisio technology-produced water leads to decreased tissue damage in the mouse model of DSS-induced acute colon inflammation partially by increasing the antioxidant capacity of the tissues. Our finding that Koisio technology-produced water significantly attenuated colon inflammation-induced increases in the MPO activity and EPO activity has also suggested important mechanisms underlying the effects of Koisio technology-produced water on the tissue injuries. It is warranted to conduct future studies to further investigate the mechanisms underlying the effects of Koisio technology-produced water on the inflammation-induced tissue damage.

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Figure Legends:

Figure 1. Koisio technology-produced water significantly attenuated colon inflammation-induced shortening of colon in the mouse model of DSS-induced acute colon inflammation. Mice drank regular drinking water or Koisio technology-produced water for 10 day, then administered with 3% (w:v) Dextran Sulfate Sodium salt (DSS) in dH_2O or Koisio Technology-produced water for 7 – 10 days. Colon lengths were then determined. ***, $P < 0.001$. N = 18 - 31.

Figure 2. Koisio technology-produced water significantly attenuated colon inflammation-induced increase in DAI in the mouse model of DSS-induced acute colon inflammation. Mice drank regular drinking water or Koisio technology-produced water for 10 day, then administered with 3% (w:v) Dextran Sulfate Sodium salt (DSS) in dH_2O or Koisio Technology-produced water for 7 days. DAI was then determined. ***, $P < 0.001$. N = 18 - 31.

Figure 3. Koisio technology-produced water significantly attenuated colon inflammation-induced increase in Spleen Index in the mouse model of DSS-induced acute colon inflammation. Mice drank regular drinking water or Koisio technology-produced water for 10 day, then administered with 3% (w:v) Dextran Sulfate Sodium salt (DSS) in dH_2O or Koisio Technology-produced water for 7 – 10 days. Spleen index was then determined. ***, $P < 0.001$. N = 18 - 31.

Fig. 4. Koisio technology-produced water significantly attenuated the increases in MPO activity in the mouse model of DSS-induced acute colon inflammation. DSS induced significant increases in MPO activity, which was significantly attenuated in the mice that had drunk Koisio technology-produced water. ***, $P < 0.001$. N = 11 - 20.

Fig. 5. Koisio technology-produced water significantly attenuated the increases in EPO activity in the mouse model of DSS-induced acute colon inflammation. DSS also induced significant increases in EPO activity, which was significantly attenuated in the mice that had drunk Koisio technology-produced water. ***, $P < 0.001$. N = 12 - 20.

Figure 1

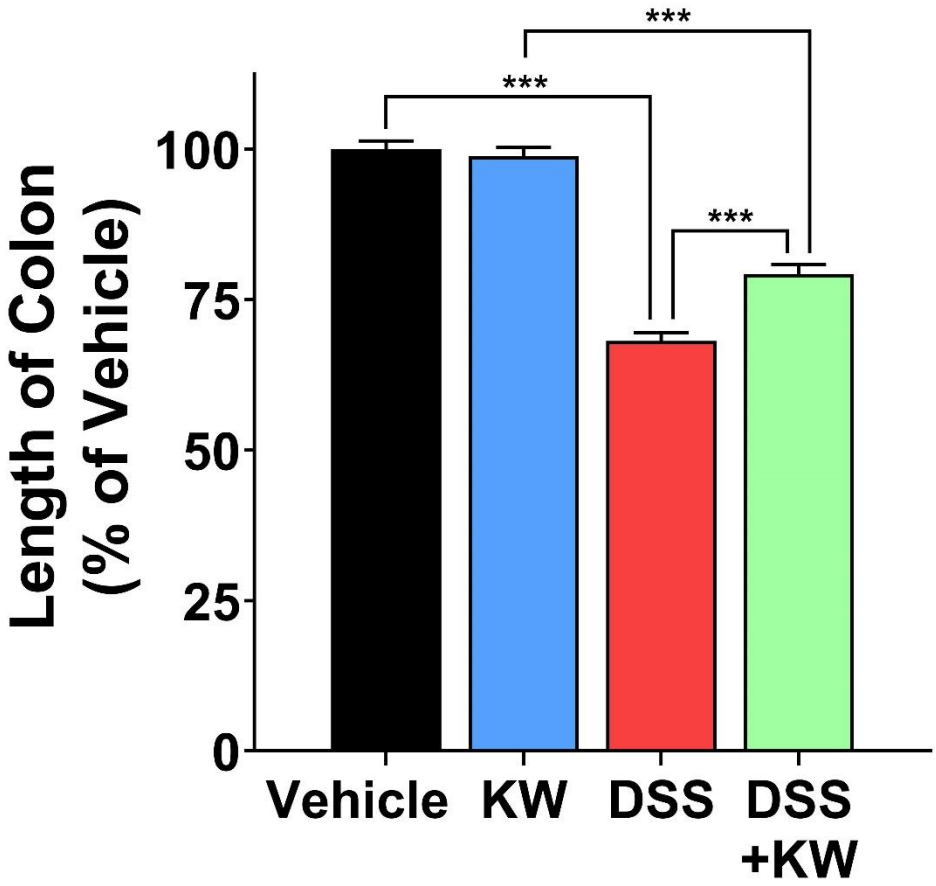

Figure 2

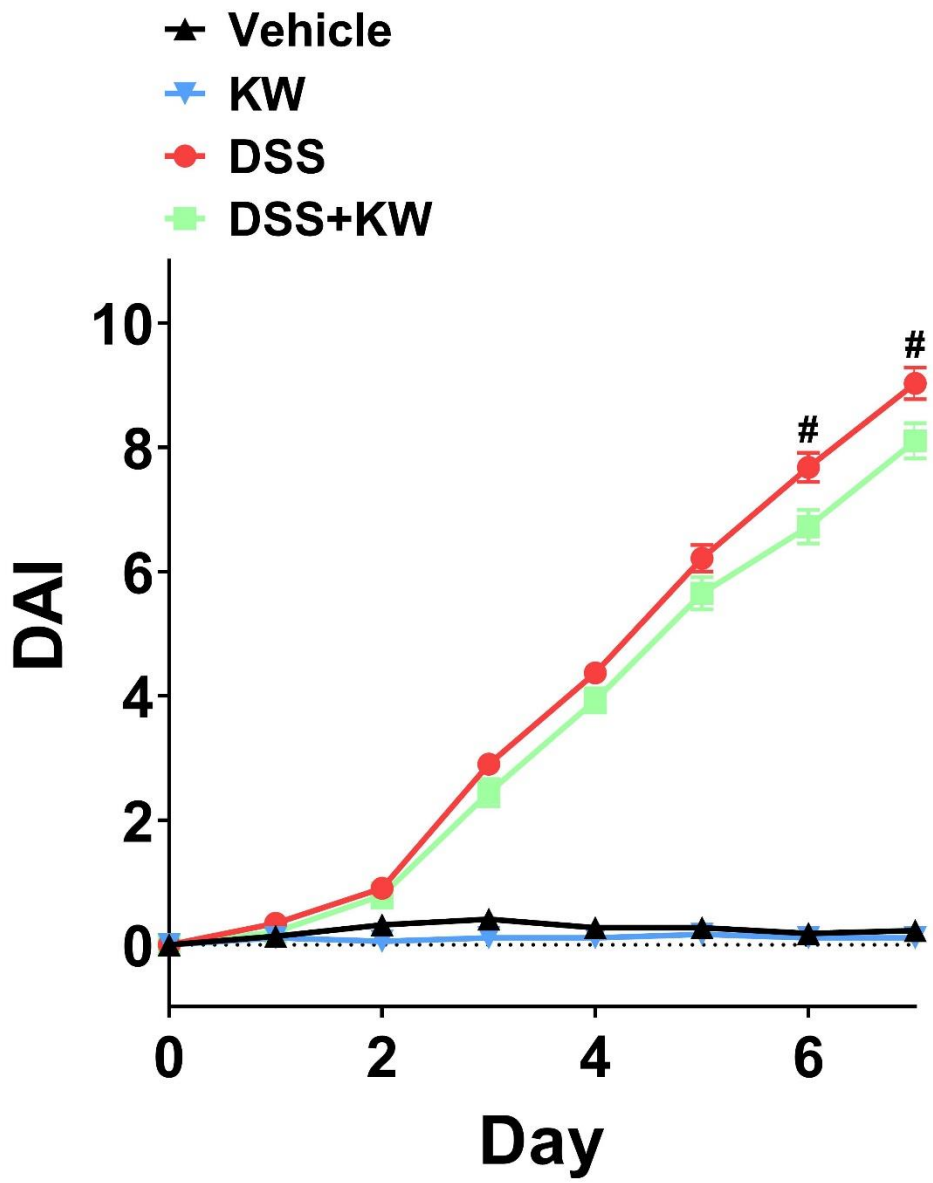

Figure 3

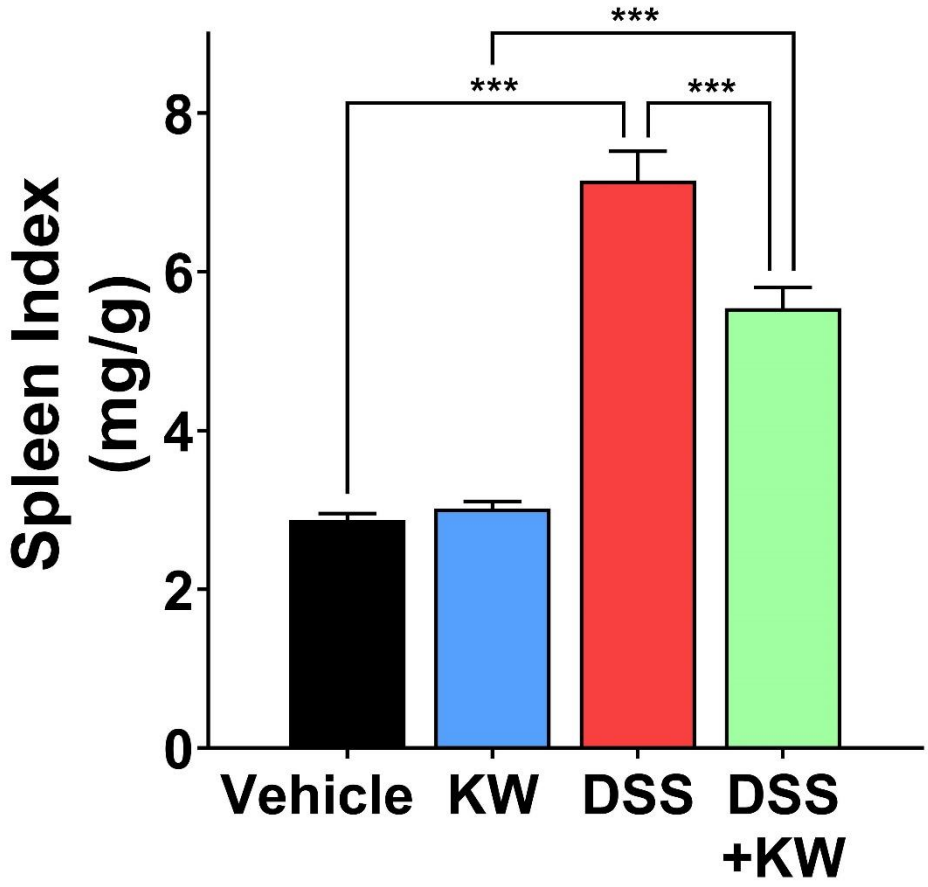

Figure 4

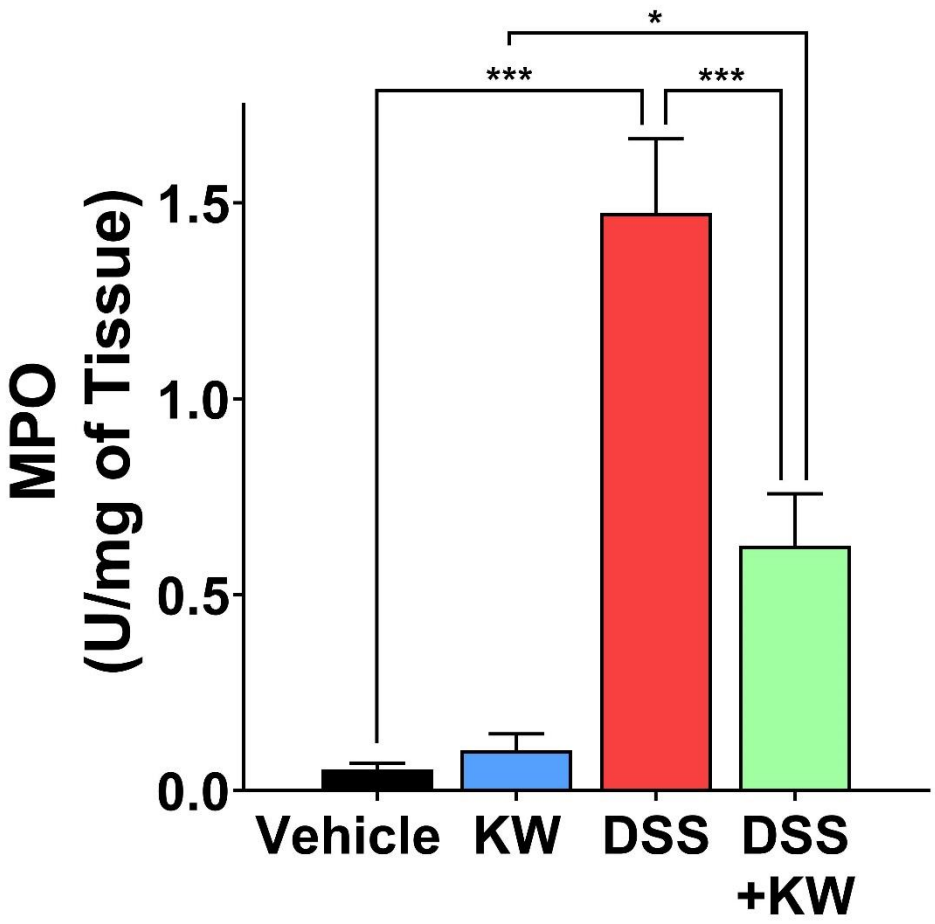

Figure 5